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# PROCEDURE FOR THE SYNTHESIS OF DIPEPTIDES OF FATTY CHAIN $N^{\alpha}$-ACYLARGININE AND PROTEIN HYDROLYZATES AS IONIC SURFACTANTS WITH ANTIMICROBIAL ACTION

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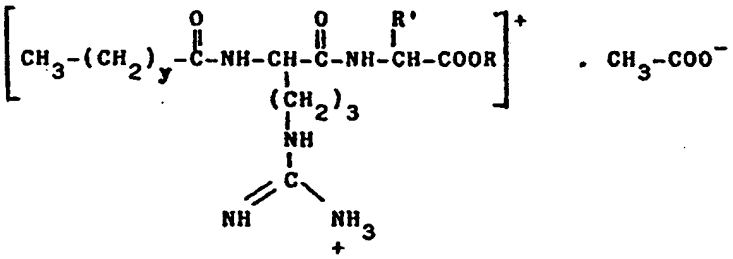
## Description

The present invention refers to the preparation of ionic surfactant molecules whose ionic group is a dipeptide constituted by arginine and another amino acid from a protein hydrolyzate, and whose hydrophobic group is a fatty acid condensed to the arginine's α-amino group.

## General Formula

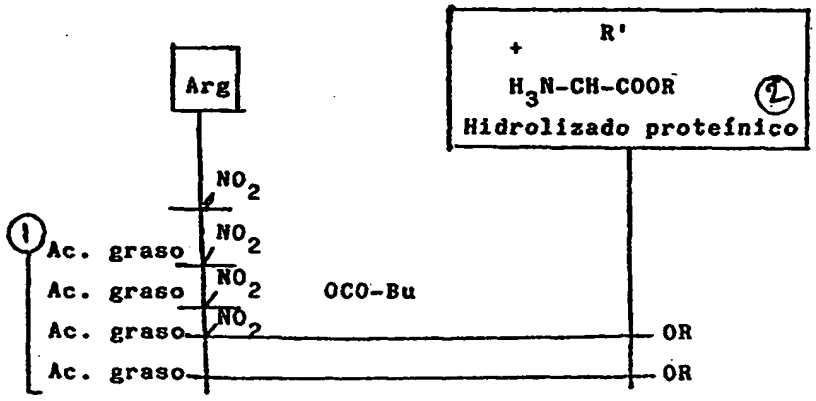


Where

- y varies from 8 to 14
- R is a short-chain alkyl residue or a monocation
- R' is a side chain of an acidic, basic, or neutral amino acid, whose proportion in the final product depends on the amino acid composition of the starting protein.

The ionic nature of these molecules depends on the groups R and R'. They may be cationic or amphoteric if R is an alkyl residue or a monocation, respectively, except if R' is an amino acid side chain.

They are obtained through the condensation of a derivative of fatty chain $N^{\alpha}$-acylarginine and a protein hydrolyzate using the mixed anhydride as a method of condensation, as indicated in the following outline:




Key: 1 Fatty acid
2 Protein hydrolyzate

The protein hydrolyzates that are used in the present note come from bird feathers and collagen and, in general, from any waste protein.

The present invention encompasses, among other things, the preparation of the following compounds:

1. $N^{\omega}$-nitroarginine
2. $N^{\alpha}$-decyl, $N^{\omega}$, dodecyl, $N^{\alpha}$-tetradecyl, or $N^{\alpha}$-hexadecylnitroarginine
3. Protein hydrolyzates
4. Alkyl esters of protein hydrolyzates
5. Dipeptides of $N^{\alpha}$-decyl, $N^{\alpha}$-dodecyl, $N^{\alpha}$-tetradecyl, or $N^{\alpha}$-hexadecylnitroarginine
6. Dipeptides of $N^{\alpha}$-decyl, $N^{\alpha}$-dodecyl, $N^{\alpha}$-tetradecyl, or $N^{\alpha}$-hexadecylarginine

The importance of this synthesis lies in the surfactant and antimicrobial properties of the final product and in its natural origin compatible with the protein structures of human skin.

Description of the method

In the described procedure, the following stages can be distinguished:

1. Preparation of fatty chain $N^{\alpha}$-acyl-$N^{\omega}$-nitroarginine from the fatty acid chloride and from $N^{\omega}$-nitroarginine in an aqueous medium whose pH is maintained between 9 and 10 with NaOH.

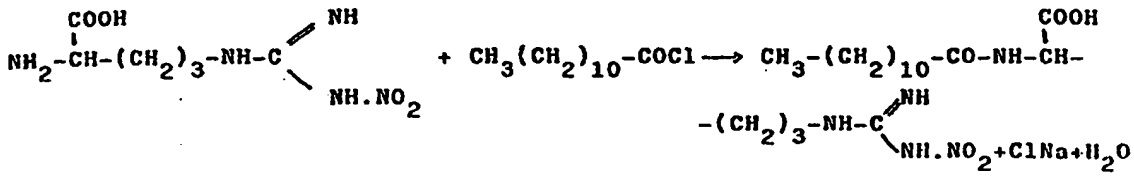
2. Preparation of the protein hydrolyzate as a mixture of free or esterified amino acids. In the first case hydrolysis of the protein is carried out with hydrochloric acid, and in the second case said protein hydrolyzate is esterified in a dry medium of methanol/HCl.

3. Preparation of dipeptides of $N^{\alpha}$-acylarginine by condensation of $N^{\alpha}$-acyl, $N^{\omega}$-nitroarginine with the protein hydrolyzate, and final elimination of the $N^{\omega}$-nitro group by hydrogenolysis in the presence of Pd/C. The mixed anhydride method was used to carry out the coupling, using isobutyl chloroformate as a reagent.

By way of example, and without thereby limiting the procedure, we will describe in detail the synthesis of dipeptides derived from $N^{\alpha}$-lauroyl-L-arginine and from the collagen hydrolyzate.

Synthesis of dipeptides from $N^{\alpha}$-lauroyl-L-arginine and collagen hydrolyzates

1. Preparation of $N^{\alpha}$-Lauroyl-L-Nitroarginine



To a solution of 1g of L-nitroarginine and 9.14 mL of 0.5N NaOH, 1.08 mL of lauroyl chloride and 4.6 mL of 1N NaOH are added cold, so that the pH is maintained between 9 and 10. The reaction mixture is kept under cold agitation until the pH drops to 7-8. The obtained solid is washed several times with ethyl ether, then with 0.2N HCl, and finally with water. Once the white solid is dry, it is crystallized from ethanol/water, yielding a solid that melts at m.p. 177-179°C with a yield of 41.53%.

$[\alpha]^{D}_{20°C} = +3.6 \pm 0.5$ (c = 1, MeOH)

Elemental analysis

| | C | H | N |
|---|---|---|---|
| Theoretical | 53.86 | 8.73 | 17.46 |

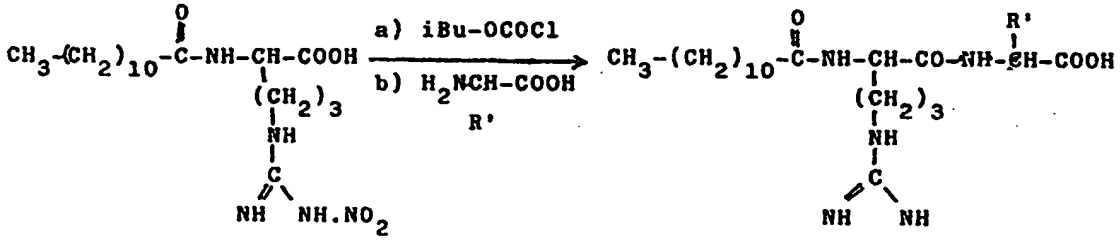
| | | | |
|---|---|---|---|
| Found | 54.23 | 8.78 | 17.19 |

2. Preparation of the collagen hydrolyzate

One gram of collagen from pieces of leather is suspended in 25 mL of HCl in a hydrolysis tube. It is closed with a nitrogen atmosphere and maintained at 105°C during 24 h.

The qualitative and quantitative analysis of amino acids is done with an amino acid autoanalyzer.

3. Preparation of dipeptides from $N^{\alpha}$-lauroyl-L-arginine




To a solution formed by 0.800 g of $N^{\alpha}$-lauroylnitroarginine [sic] and 40 mL of dimethylformamide, 0.22 mL of N-methyl morpholine are added. Then the reaction mixture is cooled to -15°C, 0.26 mL of isobutyl chloroformate is added and, at the same temperature, the mixture is kept in agitation for 90 sec. Immediately thereafter a suspension of 0.300 g of collagen hydrolyzate in 40 mL of dimethylformamide, previously neutralized with 0.25 mL of N-methylmorpholine, is also added cold.

The reaction mixture thus prepared is kept in agitation at -15°C for 1 h, after which it is allowed to reach ambient temperature and it is agitated for 4 more hours.

It is evaporated until dry and treated with a mixture of ethyl acetate/water (50%). The organic phase is washed with water, dried over anhydrous $Na_2SO_4$, and evaporated dry, yielding a sticky solid that does not crystallize and that weighs 0.670 g.

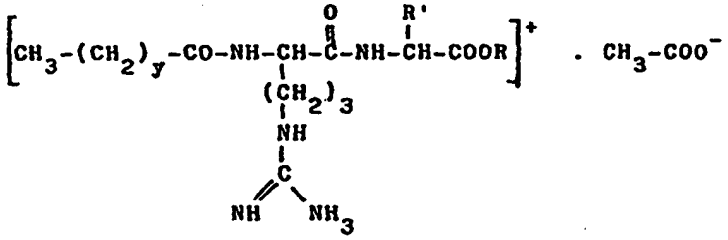
0.570 g of the previous dry solid is dissolved in 15 mL of methanol with a content of glacial acetic acid of 10%. In the presence of 0.200 g of palladium/C, hydrogen is bubbled over 8 h. Later the mixture is filtered, evaporated dry, and a solid weighing 0.320 g that does not crystallize in MeOH/HCl 0.6N/Ether is obtained. By the analysis that was conducted, it corresponds to the compounds of the present patent.

The absence of free amino acids and $N^{\alpha}$-lauroylarginine is monitored by an amino acid autoanalyzer.

Claims

The following is claimed as a new invention of the exclusive ownership and commercial use of:

1) "PROCEDURE FOR THE SYNTHESIS OF DIPEPTIDES OF FATTY CHAIN $N^{\alpha}$-ACYLARGININE AND PROTEIN HYDROLYZATES AS IONIC SURFACTANTS WITH ANTIMICROBIAL ACTION" of the general formula:



where

- y varies from 8 to 14
- R is a short-chain alkyl residue or a monocation
- R' is a side chain of acidic, basic, or neutral amino acid, whose proportion in the final product depends on the amino acid composition of the starting protein,

characterized in that in a first stage the fatty chain $N^{\alpha}$-acyl, $N^{\omega}$-nitroarginine is prepared from the fatty acid chloride and from the $N^{\omega}$-nitroarginine; in a second stage the protein hydrolyzate is prepared in the form of free amino acids by hydrolysis of the protein with HCl, or in the form of esterified amino acids by esterification of the previous protein hydrolyzate in a dry medium of methanol/HCl; and in a third stage the condensation of the $N^{\alpha}$-acyl, $N^{\omega}$-nitroarginine with the protein hydrolyzate is carried out with the mixed anhydride method using isobutyl chloroformate, and the final elimination of the $N^{\omega}$-nitro group is carried out by hydrogenolysis in the presence of Pd/C.

2) A procedure according to Claim 1, characterized by the use of L-arginine, D-arginine, or DL-arginine as the starting amino acid.

3) A procedure according to Claim 1, characterized in that it uses the nitro group as a protector of the arginine's guanidine group.

4) A procedure according to Claim 1, characterized in that it uses chlorides of pure fatty acids or a mixture thereof in a 8-14 [carbon] chain as acylating agents of the nitroarginine in a cold aqueous medium of pH 9-10.

5) A procedure according to Claim 1, characterized in that it uses waste protein hydrolyzates in the form of free or esterified acid to form the dipeptides of fatty chain $N^{\alpha}$-acylarginine.

6) A procedure according to Claim 1, characterized in that the condensation of the $N^{\alpha}$-acylnitroarginine with the protein hydrolyzate, to obtain the dipeptides of $N^{\alpha}$-lauroylnitroarginine, takes place through the formation of a mixed anhydride as follows: during 90 sec isobutyl chloroformate and $N^{\alpha}$-acylnitroarginine (previously neutralized with N-methyl morpholine) are mixed equimolecularly into dimethylformamide at -15°C; then the protein hydrolyzate, previously neutralized with N-methylmorpholine, is added to this mixture, and it is left in agitation for a max. of 4 h at ambient temperature.

7) A procedure according to Claims 1 and 6, characterized in that the purification of the dipeptides of $N^{\alpha}$-acylnitroarginine is carried out easily by extractions of ethyl acetate washed with water.

8) A procedure according to Claim 1, characterized in that deprotection of the nitro group in the final condensate, and therefore the obtainment of the $N^{\alpha}$-acylarginine dipeptides, takes place with hydrogen gas in the presence of Pd/C and in a medium of methanol containing acetic acid.

9) "PROCEDURE FOR THE SYNTHESIS OF DIPEPTIDES OF FATTY CHAIN $N^{\alpha}$-ACYLARGININE AND PROTEIN HYDROLYZATES AS IONIC SURFACTANTS WITH ANTIMICROBIAL ACTION," as described in the body of this note and claims, which consists of 7 pages written on one side.

Madrid, February 11, 1986

[signature]

[seal: Higher Council of Scientific Research]